Effects of Cortical Serotonin Depletion Induced by 3,4-Methylenedioxymethamphetamine (MDMA) on Behavior, Before and After Additional Cholinergic Blockade

Terry E. Robinson, Ph.D., Edward Castañeda, Ph.D., and Ian Q. Whishaw, Ph.D.

Repeated treatment with high doses of 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") produces a long-lasting depletion of brain serotonin, presumably because of the degeneration of serotonin axon terminals. However, very little is known about the long-term behavioral consequences of MDMA neurotoxicity. The experiments reported here were designed to evaluate the effects of MDMA neurotoxicity on a number of behavioral tests known to be sensitive to neocortical and hippocampal damage. Also, the effect of additional cholinergic blockade in MDMA-pretreated rats was evaluated because loss of both the serotonergic and cholinergic inputs to the cortex produces a functional decortication and a behavioral syndrome reminiscent of human global dementia. Partial depletion of neocortical serotonin (72.6%) did not produce deficits on a variety of behavioral tests, including a place navigation learning-set task, skilled forelimb use, or the ability to make complex judgements regarding the stimulus properties of food in a foraging situation, and neither did additional cholinergic blockade. MDMA-pretreated rats had a mild impairment in rapidly developing an efficient search strategy in the place navigation task, but once the goal was located, MDMA pretreated rats performed at control levels and showed no deficits in memory for spatial location. It is concluded that the extent of serotonergic denervation produced by MDMA is not sufficient to produce marked and lasting behavioral deficits, possibly because of neurocompensatory changes in the remaining serotonin terminals. ***[Neuropsychopharmacology 8:77–85, 1993]***

KEY WORDS: *Neurotoxicity; Learning; Amphetamines; Atropine; 5-Hydroxytryptamine*

MDMA (3,4-methylenedioxymethamphetamine; "ecstasy") is a synthetic amphetamine derivative that is of interest both because it is used recreationally (Peroutka 1987), and because it is potentially neurotoxic (Stone et al. 1986; Commins et al. 1987; Schmidt 1987). In addition to its psychomotor stimulant effects, MDMA also shares with the amphetamines the ability to deplete brain monoamines, at least when given in high doses (Seiden and Ricaurte 1987). The serotonin depletion produced by MDMA is very persistent, lasting for months, and appears to be due to the degeneration of serotonin axon terminals (Commins et al. 1987; O'Hearn et al. 1988). Although there have been a number of studies concerning the biochemical and histochemical effects of MDMA and the mechanisms underlying its neurotoxic potential, there have been very few studies

From the Departments of Psychology, The University of Michigan (TER), Arizona State University (EC), and the University of Lethbridge (IQW).

Address correspondence to Dr. Terry E. Robinson, Neuroscience Laboratory Building, The University of Michigan, 1103 East Huron Street, Ann Arbor, MI 48109.

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on the long-term behavioral consequences of MDMA neurotoxicity. Obviously, it is important to determine whether the damage to serotonin terminals produced by MDMA has behavioral consequences, and the nature of any behavioral deficits.

It was recently reported that MDMA may result in lasting neuropsychiatric dysfunction in recreational MDMA users (McCann and Ricaurte 1991), but the limited evidence from animal studies suggests MDMA neurotoxicity is not accompanied by significant deficits in tests of relatively reflexive behavior or maze learning (Slikker et al. 1989). The purpose of the experiments reported here, therefore, was twofold. First, experiments were designed to evaluate the performance of rats depleted of forebrain serotonin by MDMA pretreatment on behavioral tests known to be sensitive to damage to the hippocampus, motor cortex, and limbic cortex. These tests included a spatial navigation learning-set task (Whishaw 1985a,b), evaluation of skilled forelimb use (Whishaw et al. 1986), and an evaluation of the ability of animals to make complex judgements regarding the stimulus properties of food in a foraging situation (Whishaw and Oddie 1989; Whishaw et al. 1990). Second, it is known that serotonin depletion alone does not produce marked abnormalities in electrographic activation of the cortical mantle, but if forebrain serotonin depletion is combined with cholinergic blockade, rats are totally unable to activate the hippocampus or neocortex (i.e., to produce hippocampal rhythmical slow activity or neocortical low-voltage fast activity; Vanderwolf and Baker 1986; Vanderwolf et al. 1989). This results in functional decortication and a behavioral syndrome analogous to human global dementia (Dickson and Vanderwolf 1990; Nilsson et al. 1988; Vanderwolf 1987; Vanderwolf and Baker 1986). Therefore, we also designed experiments to evaluate the behavioral effects of cholinergic blockade with atropine sulfate in rats depleted of forebrain serotonin by MDMA pretreatment.

METHODS

Animals

Eighteen adult male Sprague-Dawley rats weighing between 200 and 220 g when the study began were used. They were housed in pairs in hanging wire mesh cages in an animal colony lighted on a 12:12-hour light/dark cycle. All behavioral testing was conducted during the light portion of the light/dark cycle. For experiments that required food deprivation, animals were reduced to 90% normal body weight and received once-a-day supplemental feeding of a measured amount of food to maintain body weight at this level.

The rats were divided randomly into two groups. The MDMA pretreated group (n = 6) received one intraperitoneal injection of 10 mg/kg of MDMA every 12 hours, for a total of eight injections. A similar treatment regimen has been shown to deplete forebrain serotonin (Ricaurte et al. 1985; Stone et al. 1986). Control rats (n = 12) received an equivalent volume of isotonic saline. Two days following the last injection of MDMA or saline the rats were placed on a food deprivation schedule, and training began on the behavioral tasks described below, which were administered in the following order: (1) spatial navigation; (2) skilled reaching; and (3) foraging.

Spatial Navigation Task

The test apparatus consisted of a round white swimming pool 146 cm in diameter and 45 cm high, which was filled to a depth of 25 cm with water (18°C) that was rendered opaque by the addition of 1,000 cc of powdered skim milk (see Whishaw 1985a,b for a more complete description). Rats could escape from the water by swimming to a platform (11 × 12 cm) that was placed 1 cm below the water surface. The platform was not visible to a swimming rat and could only be located by reference to surrounding room cues. The room itself was dimly lit (roughly 10 lux). Four points on the pool rim were designated as north (N), south (S), east (E), and west (W); on this basis, the pool surface was divided into four quadrants of equal area, NE, NW, SE, and SW. The time it took animals to swim from a start point (see below) to the platform was timed with a stop watch, and swim patterns were drawn on a schematic diagram of the swimming pool. If a rat swam directly to the platform, staying within an 18-cm-wide path from the start point to the platform, its performance was scored as correct. If it deviated from this route at any point an error was recorded for that trial.

Animals were initially trained in this apparatus on a type of learning-set task similar to that described previously (Whishaw 1985a,b). Briefly, the task required animals to learn a new problem (place) each day for three successive days. On the first day, the platform was placed randomly in one of six possible locations. Each rat was initially placed in the water facing the wall of the pool at one of the major compass points (N, S, E, or W), and allowed to swim until it found the hidden platform. After climbing onto the platform it was left for 5 seconds before it was removed and a second trial given immediately from the same start point as the previous trial. At the end of the second trial, each rat was returned to a holding cage for approximately 5 minutes. Each rat then received a second pair of trials from a new starting location, and testing continued in this manner until it had received two trials from each of the four starting locations, for a total of eight trials per day. This procedure was repeated for 3 consecu-

tive
form location on each day.

To perform well in this task, animals must acquire the learning "set" i.e., they must learn that on any particular day, (1) there is a platform in the pool, and (2) its location is constant on that day. Once they master the set, they solve this problem in a single trial. That is, on the first trial of a given day they first search for the platform at its old location, and not finding it there they then search for its new location. On all subsequent trials they return directly to the new location, thus learning each new place in one trial.

On the fourth day of testing, the procedure described above was modified. First, the platform was left in the same location as on the previous day (day 3), and each rat received one trial from each of the four start points. This test evaluated their memory for the previous platform location over a 24-hour interval. Next, each rat received an injection of 50 mg/kg of atropine sulfate; 30 minutes later they received four pairs of trials from each start point, again with the platform in the same location as on day 3. This test evaluated the animal's memory for the place while they were subjected to cholinergic blockade.

At the end of the atropine test, the animals were allowed 2 days to recover. After this they were tested for 3 more consecutive days exactly as described for days 1 through 3, but with a different platform location each day (learning set: days 7 to 9). This test was intended to reevaluate the ability of the animals to perform the learning set after having a total of 4 days' experience in the swimming pool.

Skilled Reaching Task

After completion of testing for spatial navigation, the rats were trained to reach with their paws to retrieve food in an apparatus described in detail previously (Whishaw et al. 1986). Briefly, the test chamber had three solid walls constructed from plexiglas and a front wall constructed of 2-mm-diameter vertically-oriented cylindrical bars separated from each other by 9 mm edge to edge. A 4-cm-wide and 5-mm-deep tray containing granules of food (20 to 40 mg chick feed) was mounted directly in front of the test chamber at the level of the floor, and extended for the length of the front wall. To obtain food, a rat had to reach through the space between any two bars, grasp a piece of food, and retract it. The test chamber had a metal grid floor, so if a rat dropped a piece of food it fell through the grid and was lost.

Food-deprived rats were trained to reach for food in this apparatus for 1 hour a day for 7 consecutive days, with the food tray located 10 mm from the front wall. After this they were given three separate tests: (1) in the first test, the food tray was located 10 mm from the front wall (inside edge of the tray to outside edge of the bars) and reaching performance was scored for 10 minutes; (2) the next day, the animals were placed into the apparatus but the food tray was pulled back from the edge of the cage to a distance of 20 mm; reaching performance was again scored for 10 minutes; and (3) on the third day, the rats were given an intraperitoneal injection of atropine sulfate (50 mg/kg); 30 minutes later they were given an additional 10-minute reaching test with the food tray located 20 mm from the front wall.

Performance was scored by depressing buttons connected to a microcomputer, indicating "hits" and "misses" for each limb. Each attempt to retrieve food, defined as insertion of a paw through the bars of the cage, was scored as a "reach." If the rat obtained a piece of food and then consumed it, the reach was scored as a hit; otherwise the reach was scored as a miss. Accuracy was calculated as the percent of reaches resulting in a hit.

Foraging Task

The foraging apparatus consisted of a wire mesh home cage attached to a Plexiglas alley, which had a food source at its far end (Whishaw and Oddie 1989; Whishaw et al. 1990). The mesh cage was 21 cm wide, 25 cm high, and 19 cm long, with a metal roof and one Plexiglas side through which an animal could be observed. A 6-cm-wide by 7.5-cm-high door allowed access to the alley, which was 60 cm long, 25 cm wide, and 26 cm high and constructed entirely from clear Plexiglas. The animals always had free access to the alley from the home cage.

During a 3-day habituation phase, the animals were placed in the apparatus for 1 hour per day in groups of six. The pretraining phase followed, during which time each animal was placed individually into the home cage. If it walked to the far end of the alley it was given a food pellet through a 1-cm-diameter hole in the far wall. The food pellets (Bioserve Inc.) weighed 20, 37, 45, 75, 94,190, 300, 500, 750, or 1,000 g. Hereafter, they are referred to as pellets size 1 to 10, respectively. Pretraining continued for 15 minutes per day for a further 3 days.

Following pretraining, all rats showed stable performance in this apparatus, and they then received four formal tests on 4 consecutive days. Each test consisted of 10 trials, with each trial consisting of presentation of one of the 10 different sized food pellets. The order of presentation of the pellets was determined randomly. Behavior was scored as follows: 1) *eat* indicated that a rat swallowed the food pellet immediately on receipt at the food source; 2) *sit* indicated that on receipt of a food pellet, the rat transferred it from its mouth to its paws and sat back on its haunches, eating the food from its paws; and 3) *carry* indicated that a rat carried a food

pellet back to the home cage. In addition, the duration of three food handling behaviors was recorded: 1) *carry time* consisted of the time required to carry a food pellet from the point of receipt back to the home cage, on those trials when a pellet was carried; 2) *eat time* was the time required to consume a food pellet following receipt, either at the food source or in the home cage; and 3) *return time* the time required to return to the food source after carrying the food pellet to the home cage (timing started immediately after consumption of the pellet).

The rationale for including this task in the present study is that it requires rats to make complex judgements about the stimulus properties of food, and, on the basis of this evaluation, reach a decision about whether a food pellet should be eaten immediately on receipt, out in the open, or whether it should be carried back to the safety of the home cage (Whishaw, 1990; see below). This complex decision-making process is known to be disrupted by damage to a variety of forebrain structures (Whishaw et al. 1990), and therefore it was hypothesized that it may be sensitive to cognitive impairments associated with MDMA neurotoxicity.

Assay for Monoamines

At the end of all behavioral testing, 35 to 40 days after MDMA pretreatment, each rat was killed by decapitation and its brain was rapidly removed and placed in ice-cold saline. After it cooled (30 to 45 seconds) it was placed in a chilled cutting block and brain slices were obtained through the caudate putamen (Heffner et al. 1980). Samples of the caudate nucleus were obtained with a micropunch. In addition, the entire neocortex was dissected and the caudate and neocortex tissue samples were placed into individual tubes containing 0.05 N perchloric acid and dihydroxybenzylamine (internal standard). The samples were homogenized and centrifuged at 1500*g* for 4 minutes. The supernatant was filtered and assayed by HPLC and electrochemical detection using procedures similar to those described previously (Robinson et al. 1987).

RESULTS

Neurochemistry

As expected, MDMA pretreatment produced a significant decrease in the tissue concentration of serotonin in both the neocortex and caudate nucleus. The concentration of serotonin in the neocortex of MDMA-pretreated rats was depleted by 72.6% relative to control animals (0.36 ± 0.01 vs. 1.33 ± 0.05 ng/mg, respectively; $t_{16} = 12.1$, $p < 0.001$). In the caudate nucleus, serotonin was only depleted by 32.3% (0.87 ± 0.07 vs. 1.28 ± 0.13 ng/mg; $t_{16} = 4.23$, $p = 0.056$). There was no significant effect of MDMA pretreatment on the concentration of dopamine (DA) in either the caudate or neocortex (MDMA values were 110% and 119% of control, respectively).

Spatial Navigation Task

Figure 1A illustrates the ability of MDMA-pretreated and control rats to acquire the initial learning set over the first 3 days of training, when the rats received a new place problem each day. There was a significant effect of MDMA pretreatment on the time required to find the hidden platform relative to controls (effect of group, $F_{1,16} = 8.18$, $p < 0.011$). However, both the control and MDMA-pretreated rats did learn the problem, as indicated by a significant decrease in latency across trials from 20 to 60 seconds on the first trial to less than 10 seconds by the eighth trial (effect of trials, $F_{7,112} = 24.3$, $p < 0.001$). The greatest improvement took place between the first and second trial, and the MDMA-pretreated group showed elevated latencies only during the first few trials (group by trials, $F_{7,112} = 3.48$, $p < 0.002$; Fig. 1A). Thus, MDMA-pretreated rats were less efficient than control animals, in adopting an efficient search strategy when the platform was in a new location, as indicated by especially long swim latencies on the first few trials. Nevertheless, the animals were able to learn the location of the platform and eventually perform at control levels, as indicated by latencies of about 5 seconds in both groups by the seventh to eighth trial (Fig. 1A).

For the first four trials on the fourth day of testing, the platform was located in the same place as on the previous day to test for retention of place information over a 24-hour interval. Both groups had swim latencies of less than 10 seconds, and there were no group differences (group $F_{1,16} < 1$), indicating that MDMA pretreatment did not impair memory for place over a 24-hour test-retest interval (Fig. 1B). The animals were then given atropine to produce cholinergic blockade, and 30 minutes later given an additional eight trials with the platform in the same place. Atropine slightly impaired performance in both groups, as is typically observed (Whishaw 1985a), but again, there were no significant group differences in either latencies or errors (Fs < 1; Fig. 1B).

Fig. 1C illustrates the ability of the animals to reacquire the learning-set task over an additional 3 days of testing, using a new platform location on each day, after having had 4 days of experience in the swimming pool. There were no differences between the MDMA-pretreated and control groups on this retest for either swim latency (Fig. 1C) or errors (data not shown). It can be seen in Fig. 1C that 1) both groups adopted efficient search strategies, as indicated by relatively low swim latencies on trial 1 (compare the latencies for trial

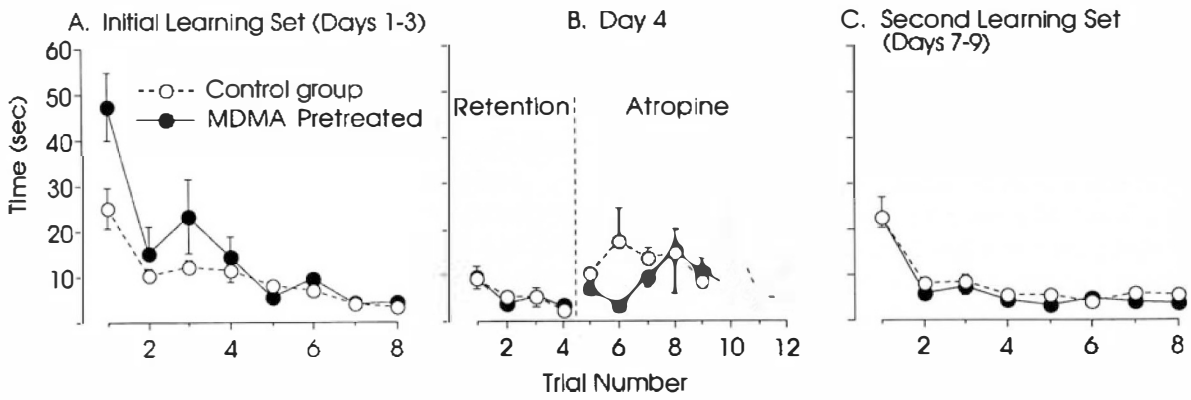

Figure 1. The mean (± SEM) latency to swim from the start point to a hidden platform in control (saline-pretreated) and MDMA-pretreated rats. A: Performance on the initial learning-set task. Data for each trial were averaged over the first 3 days of training. The MDMA-pretreated group had significantly longer latencies than control animals during the first few trials, but performed at control levels over trials 4 through 8. B: Performance on day 4. There was no difference between the groups on the initial retention test or following treatment with atropine. C: Performance when retested on days 7 through 9. The two groups did not differ significantly.

1 in Fig. 1A vs. 1C); 2) both groups acquired the learning set, as indicated by essentially asymptotic performance by trial 2; and 3) both groups showed rapid acquisition of knowledge regarding the location of the platform, as indicated by swim latencies of about 5 seconds between trials 2 to 8 (Fig. 1C).

Skilled Reaching Task

Rats in the control and MDMA-pretreated groups performed at a comparable level in the skilled reaching task (Table 1). The two groups did not differ in the number of attempts to retrieve a food pellet at either the 10- or 20-mm distance (Table 1). In addition, MDMA pretreatment did not decrease accuracy (hit percent) and the MDMA-pretreated group was actually somewhat more accurate than control animals at the 20-mm distance, (F_{16} = 15.8, $p < 0.01$). Performance declined in both groups under the influence of atropine sulfate, but rats in both groups continued to reach, and they achieved the same level of accuracy (Table 1). The changes in reaching seen in both groups under atropine were attributable to sluggish performance and very slow eating following successful reaches, rather than to an impairment in the motor skills involved in reaching.

Foraging Task

Both control and MDMA-pretreated rats showed the typical pattern of behavior seen in this task, which has been described in detail previously (Whishaw et al. 1990). There was no effect of MDMA pretreatment on the probability of "eat," "sit," or "carry" as a function of pellet size (effect of group Fs all < 1). This is illustrated in Figure 2, which shows that the probability of carrying a pellet home as a function of pellet size is exactly the same in both groups. Figure 3 shows that the time required to carry a pellet home (Fig. 3A), to eat a pellet (Fig. 3B), and to return to the food source after eating a pellet in the home cage (Fig. 3C), as a function of pellet size, was essentially the same in both groups. Furthermore, these latencies were affected by pellet size in the same manner in both groups.

Table 1. Number of Attempts to Reach for Food and Accuracy of Reaching During 10-minute Tests When Reaching a Distance of 10 or 20 mm and After Treatment with Atropine Sulfate in Control and MDMA-Pretreated Rats

	10 mm	20 mm	20 mm with Atropine
Number of Attempts			
Control	74 ± 4	90 ± 2	47 ± 5
MDMA	62 ± 3	68 ± 9	43 ± 3
Accuracy ("hit percent")			
Control	58 ± 3	37 ± 3	36 ± 7
MDMA	64 ± 4	52 ± 3*	35 ± 4

* Differs from control, t_{16} = 15.8, $p < 0.01$.

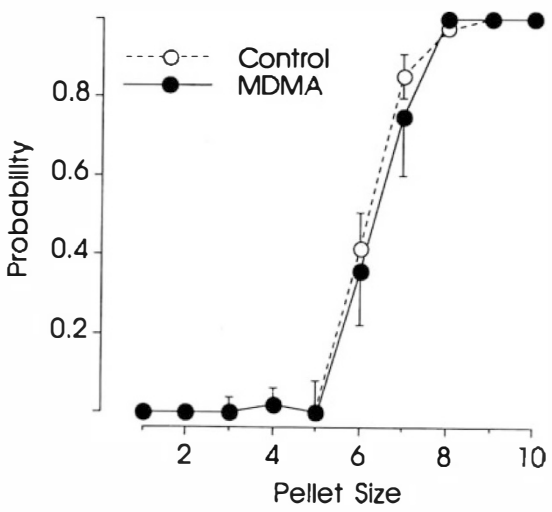

Figure 2. The mean (± SEM) probability of carrying a food pellet back to the home cage as a function of pellet size in control and MDMA-pretreated rats. All animals immediately swallowed the smallest food pellets at the food source, sat and ate the intermediate sized pellets from their paws at the food source, and all animals carried the largest food pellets back to the home cage. There was a marked increase in the probability of carrying pellets home as a function of increasing pellet size ($F_{9,144}$ = 188, $p < 0.001$), but MDMA pretreatment had no effect (Fs < 1).

DISCUSSION

MDMA pretreatment produced a 72.6% decrease in the concentration of serotonin in the neocortex relative to saline-pretreated control animals, as expected from previous reports (Stone et al. 1986; Commins et al. 1987; Schmidt 1987). However, despite a marked depletion in cortical serotonin, the performance of MDMA-pretreated rats was remarkably normal on a variety of tasks known to be sensitive to cortical damage.

Skilled reaching is impaired by damage to the motor cortex or the striatum (Whishaw et al. 1986), but the MDMA-pretreated group performed just as well as controls on this task. Perhaps even more surprising was the performance of MDMA-pretreated rats in the foraging task. This task requires animals to make a rapid decision about whether to eat food at the food source or to carry it back to the safety of the home cage and eat it there. In a series of experiments, Whishaw (1990; Whishaw et al. 1990) has shown that in making this decision, rats make judgements about the stimulus characteristics of the food (e.g., size, weight, texture, etc.), and use this information to estimate the time required to eat the food. They also estimate the time it would take to return to the home cage. If the estimated time to eat the pellet is less than the time required to return home, rats usually eat the food at the food source. But if the time required to eat the food is greater than the time required to return home, rats usually carry the pellet home and eat it there. A decision such as this obviously requires a series of complex perceptual and cognitive processes, and performance in this situation is disrupted by a variety of manipulations that affect neocortical activity, including small cortical lesions (Whishaw et al. 1990). However, rats depleted of cortical serotonin by MDMA performed normally on this task, both in terms of the probability of carrying food home as a

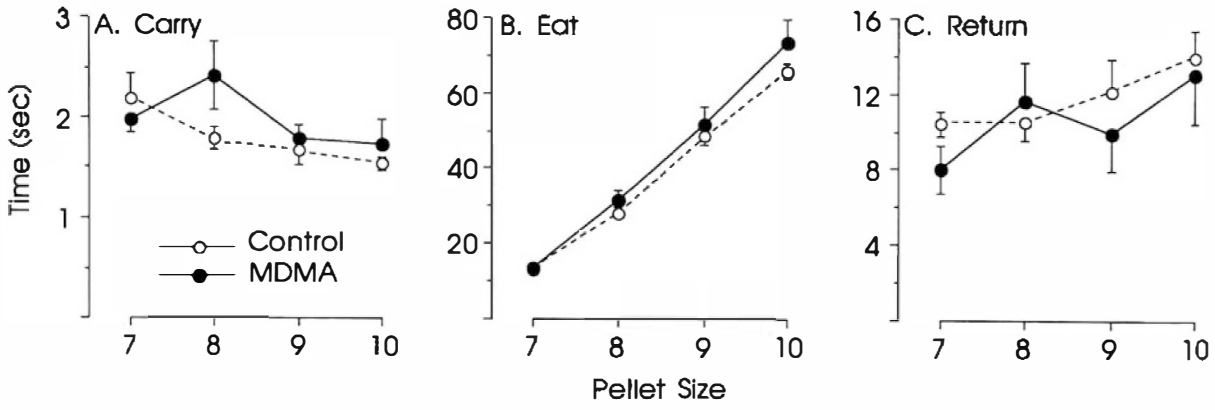

Figure 3. The mean (± SEM) latency to perform various behaviors in the foraging task as a function of pellet size in control and MDMA-pretreated rats. A: The time taken to carry a pellet from the food source to the home cage. There was a small group difference as indicated by pellet size interaction ($F_{3,48}$ = 3.36, p = 0.02; effect of group, $F_{1,16}$ = 0.53), but inspection shows this was due to a small increase in carry time in MDMA-pretreated rats only for pellet size 8. B: The latency to consume a food pellet as a function of pellet size. This increased as a function of pellet size to the same extent in control and MDMA-pretreated rats (effect of group and interaction nonsignificant; effect of pellet size, $F_{3,48}$ = 37, $p < 0.001$). C: The latency to return to the food source after eating a food pellet in the home cage. There were no group differences (effect of group, $F_{1,16} < 1.0$; interaction nonsignificant), but both groups took longer to return to the food source after consuming large pellets than after consuming small pellets (effect of pellet size, $F_{3,48}$ = 6.05, p = 0.001).

function of pellet size and of the time required to complete the various behaviors.

The only impairment observed in MDMA-pretreated rats was in the spatial navigation learning-set task, and then only on the first 3 days of training. Over the first 3 days, animals were required to learn a new place each day. MDMA-pretreated rats were significantly slower than control rats in finding the platform on the first few trials of each day. This suggests that MDMA pretreatment produced a small impairment in the ability of animals to develop an efficient search strategy when first confronted with a new problem. However, once they found the platform, they remembered its location and performed as well as controls, as indicated by very low latencies in swimming to the hidden platform during the last four to five trials on each day. This pattern of performance is similar to that obtained in rats with partial hippocampal CA1 damage induced by ischemia (Auer et al. 1989). Furthermore, after they had 4 days of experience on this task, MDMA-pretreated rats performed at control levels (Fig. 1C). They not only developed efficient search strategies, as indicated by low latencies on the first trial, but they acquired the learning set, as indicated by nearly asymptotic performance by the second trial, and they acquired knowledge of the location in space where the goal was located, as indicated by swim latencies of about 5 seconds between trials 2 and 8. MDMA-pretreated rats also showed normal retention of place information over a 24-hour period, as indicated by their performance over the first four trials on day 4 (Fig. 1B).

In summary, depletion of over 70% of neocortical serotonin produced a very mild impairment in learning, which seemed to involve only the ability to rapidly develop an efficient search strategy when initially confronted with a new place navigation problem. But once the goal was located, MDMA-pretreated rats performed at control levels and showed no deficits in memory for spatial location. The hippocampus is known to be depleted of serotonin by MDMA in a similar manner as the neocortex, and there are long-lasting alterations in glucose utilization in the hippocampus of MDMA-pretreated rats (Sharkey et al. 1991). Because the hippocampus is important for performing place navigation problems similar to those used here (Auer et al. 1989), the small impairment we saw could be due to a subtle MDMA-induced deficit in hippocampal function.

There is considerable evidence that both serotonergic and cholinergic inputs to the cortex are capable of producing cortical activation (i.e., low-voltage fast activity in the neocortex and rhythmical slow activity in the hippocampus; Vanderwolf 1988). If both of these inputs are lost (by damage or pharmacological blockade), animals show profound deficits in behavior and cognitive function reminiscent of global dementia in humans (Dickson and Vanderwolf 1990; Vanderwolf 1987). This state is presumably due to the fact that such animals are totally unable to activate the cortical mantle, and are thus functionally decorticate. We reasoned, therefore, that if partial serotonin depletion produced by MDMA pretreatment did not itself result in profound behavioral disturbances, perhaps additional cholinergic blockade would unmask deficits in behavior not seen following either MDMA pretreatment or cholinergic blockade alone. However, that was not the case. MDMA-pretreated rats given atropine showed no deficits on the skilled reaching or place navigation tasks relative to control rats given atropine. Atropine produced a mild impairment in both groups on the place navigation task and a slight decrease in skilled reaching, but neither group showed deficits reminiscent of global dementia.

There are a number of reasons why the depletion of serotonin produced by MDMA may not have resulted in any marked changes in behavior in the present study. First, the serotonin depletion produced by MDMA was incomplete, and the remaining serotonin terminals may have been able to compensate for this amount of damage. Following a comparable DA depletion in the striatum, for example, there is remarkable upregulation of DA synthesis and release that, in combination with the loss of DA uptake sites, is sufficient to normalize the extracellular concentration of DA (Robinson et al. 1990). Similar lesion-induced compensatory adaptations may also occur in serotonergic systems (Stachowiak et al. 1986).

Second, recent studies suggest that MDMA only destroys the relatively fine serotonergic axons that arise from the dorsal raphe, but spares the beaded serotonergic axons that arise from the median raphe (Mamounas and Molliver 1988; O'Hearn et al. 1988). This may be why even higher doses of MDMA than that used here do not produce an even greater depletion of serotonin (Ricaurte et al. 1985). Therefore, it is possible that an MDMA-resistant population of serotonin terminals from the median raphe, and not an MDMA-sensitive population from the dorsal raphe, is important for the behavioral and cognitive functions required to perform the tasks used here.

In summary, partial depletion of cortical serotonin produced by MDMA pretreatment did not result in any marked deficits in a variety of tasks, including skilled reaching, foraging, or a place navigation learning-set task, which is consistent with previous studies in both rats and monkeys (Slikker et al. 1989). Of special interest is the observation that additional cholinergic blockade also failed to significantly impair behavior in MDMA-pretreated rats (see Vanderwolf 1987). Nevertheless, it is probably unwise to conclude from the lack of any marked behavioral deficits in this study that MDMA pretreatment does not have persistent functional consequences. For example, although we and

others (Slikker et al. 1989) have sampled a wide range of behaviors, the behavioral analyses have been by no means exhaustive. The tasks used to date may not have been sensitive enough to detect subtle deficits in behavior, or deficits may occur in classes of behavior that have not yet been examined (e.g., in social behavior or emotional responsiveness). Finally, it is important to note that we did find that MDMA-pretreated rats were mildly impaired when initially confronted with the place navigation task. Although the deficit was not severe, MDMA-pretreated rats did take somewhat longer than normal to develop efficient strategies when faced with a new problem. A seemingly subtle impairment such as this could be biologically significant in a more natural setting, where animals are continually faced with many new spatial and social problems critical for survival on a daily basis.

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REFERENCES

Auer RN, Jensen ML, Whishaw IQ (1989): Neurobehavioral deficit due to ischemic brain damage limited to half of the CA1 sector of the hippocampus. J Neurosci 9:1641–1647

Commins DL, Vosmer G, Virus RM, Woolverton WL, Schuster CR, Seiden LS (1987): Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. J Pharmacol Exp Ther 241:338–345

Dickson CT, Vanderwolf CH (1990): Animal models of human amnesia and dementia: Hippocampal and amygdala ablation compared with serotonergic and cholinergic blockade in the rat. Behav Brain Res 41:215–227

Heffner TG, Hartman JA, Seiden LS (1980): A rapid method for the regional dissection of the rat brain. Pharmacol Biochem Behav 13:453–456

Mamounas LA, Molliver ME (1988): Evidence for dual serotonergic projections to neocortex: Axons from the dorsal and median raphe nuclei are differentially vulnerable to the neurotoxin p-chloroamphetamine (PCA). Exp Neurol 102:23–36

McCann UD, Ricaurte GA (1991): Lasting neuropsychiatric sequelae of (±)methylenedioxymethamphetamine ("ecstasy") in recreational users. J Clin Psychopharmacol 11:302–305

Nilsson OG, Strecker RE, Daszuta A, Björklund A (1988): Combined cholinergic and serotonergic denervation of the forebrain produces severe deficits in a spatial learning task in the rat. Brain Res 453:235–246

O'Hearn E, Battaglia G, De Souza EB, Kuhar MJ, Molliver ME (1988): Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: Immunocytochemical evidence for neurotoxicity. J Neurosci 8:2788–2803

Peroutka SJ (1987): Incidence of recreational use of 3,4-methylenedimethoxymethamphetamine (MDMA, "ecstasy") on an undergraduate campus. N Engl J Med 317:1542–1543

Ricaurte G, Bryan G, Strauss L, Seiden L, Schuster C (1985): Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals. Science 229:986–988

Robinson TE, Becker JB, Young EA, Akil H, Castañeda E (1987): The effects of footshock stress on regional brain dopamine metabolism and pituitary beta-endorphin release in rats previously sensitized to amphetamine. Neuropharmacology 26:679–691

Robinson TE, Castañeda E, Whishaw IQ (1990): Compensatory changes in striatal dopamine neurons following recovery from injury induced by 6-OHDA or methamphetamine: A review of evidence from microdialysis studies. Can J Psychol 44:253–275

Schmidt CJ (1987): Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. J Pharmacol Exp Ther 240:1–7

Seiden LS, Ricaurte GA (1987): Neurotoxicity of methamphetamine and related drugs. In Melzer HY (ed), Psychopharmacology: The Third Generation of Progress. New York, Raven Press, pp 359–366

Sharkey J, McBean DE, Kelly PAT (1991): Alterations in hippocampal function following repeated exposure to the amphetamine derivative methylenedioxymethamphetamine ("ecstasy"). Psychopharmacology 105:113–118

Slikker WJ, Holson RR, Ali SF, Kolta MG, Paule MG, Scallet AC, McMillan DE, Bailey JR, Hong JS, Scalzo FM (1989): Behavioral and neurochemical effects of orally administered MDMA in the rodent and nonhuman primate. Neurotoxicology 10:529–542

Stachowiak MK, Stricker EM, Jacoby JH, Zigmond MJ (1986): Increased tryptophan hydroxylase activity in serotonergic nerve terminals spared by 5,7-dihydroxytryptamine. Biochem Pharmacol 35:1241–1248

Stone DM, Stahl DC, Hanson GR, Gibb JW (1986): The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain. Eur J Pharmacol 128:41–48

Vanderwolf CH (1987): Near-total loss of "learning" and "memory" as a result of combined cholinergic and serotonergic blockade in the rat. Behav Brain Res 23:43–57 (published erratum appears in Vol. 26)

Vanderwolf CH (1988): Cerebral activity and behavior: Control by central cholinergic and serotonergic systems. Int Rev Neurobiol 30:225–340

Vanderwolf CH, Baker GB (1986): Evidence that serotonin mediates noncholinergic neocortical low voltage fast activity, non-cholinergic hippocampal rhythmical slow activity and contributes to intelligent behavior. Brain Res 374:342–356

Vanderwolf CH, Leung LW, Baker GB, Stewart DJ (1989):

The role of serotonin in the control of cerebral activity: Studies with intracerebral 5,7-dihydroxytryptamine. Brain Res 504:181–191

Whishaw IQ (1985a): Cholinergic receptor blockade in the rat impairs locale but not taxon strategies for place navigation in a swimming pool. Behav Neurosci 99:979–1005

Whishaw IQ (1985b): Formation of a place learning-set by the rat: A new paradigm for neurobehavioral studies. Physiol Behav 35:139–143

Whishaw IQ (1990): Time estimates contribute to food handling decisions by rats: Implications for neural control of hoarding. Psychobiology 18:460–466

Whishaw IQ, O'Connor WT, Dunnett SB (1986): The contributions of motor cortex, nigrostriatal dopamine and caudate-putamen to skilled forelimb use in the rat. Brain 109:805–843

Whishaw IQ, Oddie SD (1989): Qualitative and quantitative analyses of hoarding in medial frontal cortex rats using a new behavioral paradigm. Behav Brain Res 33:255–266

Whishaw IQ, Oddie SD, McNamara RK, Harris TL, Perry BS (1990): Psychophysical methods for study of sensory-motor behavior using a food-carrying (hoarding) task in rodents. J Neurosci Methods 32:123–133